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Title Page:

The impact of HPV cervical screening on negative large loop excision of the transformation zone (LLETZ): A comparative cohort study.

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Abstract:

Objective: To determine the incidence and predictors of negative large loop excision of the transformation zone (LLETZ) following the introduction of Human papillomavirus (HPV) cervical screening.

Method: A retrospective cohort study. Two independent cohorts, who attended for a LLETZ procedure, before and after the introduction of HPV cervical screening were compared. For each cohort, 401 individuals were randomly selected from a colposcopy database. Clinical and colposcopic variables were extracted. The incidence of negative LLETZ was estimated in each cohort. Regression analysis was used to adjust for potential confounders and explore predictors of negative LLETZ.

Results: Eighty women (19.9%) from the pre-HPV testing cohort and 54 women (13.4%) from the post-HPV cohort were negative for cervical intraepithelial neoplasia (RR 0.75, CI: 0.55 to 0.93). In the post-HPV testing cohort, independent predictors of negative LLETZ were low grade cytology (RR 3.60, CI: 2.18-5.97) and a type 3 transformation zone (TZ) (RR 2.88, CI: 1.76-4.72). Women with both low grade cytology and a TZ type 3 were 8.6 times more likely to have a negative LLETZ (absolute risk 40%, 95% CI: 27-54%).

Conclusions: Despite a 25% reduction in negative LLETZ following the introduction of HPV cervical screening, the incidence is still high. These results highlight the importance of continuing to improve the specificity of cervical intraepithelial neoplasia screening; this should include the use of biomarkers that detect HPV transforming infections and techniques that sample an entirely endocervical transformation zone.

Keywords: Negative LLETZ, HPV testing, unsatisfactory colposcopy

Introduction:

Loop excision of the cervical transformation zone (LLETZ) has helped reduce the overall mortality rate from cervical cancer by 60% in the UK through excision of the precursor lesion, cervical intraepithelial neoplasia (CIN).[1,2] Recent evidence however has suggested that depth of excised tissue proportionally increases the risk of preterm labour[3]. Furthermore, LLETZ has been shown to increase the risk of cervical stenosis which can cause infertility, amenorrhoea and difficulty with subsequent cytological assessments[4,5].

A negative LLETZ occurs when no CIN is identified in the histological specimen and the reported incidence in women with biopsy confirmed CIN2+ varies from 5.9% to 41%[6-11]. This large variation is secondary to differing criteria for treatment. Given that 56% of women referred to colposcopy in England are 25-35 years old, in prime reproductive age, and 12.9% of these referrals will result in a LLETZ[12], a reduction in false positive cervical screening would potentially reduce unnecessary treatments and the risk of infertility or preterm labour.

Human Papillomavirus (HPV) cervical screening was introduced in 2011 in six sentinel sites in the UK[13] following the publication of randomised control trials which demonstrated that HPV DNA detection was a more sensitive screening test than cytology for squamous cell lesions (94.6% vs 83.9%)[14,15]. Subsequently, there has been contradictory evidence regarding the potential effect of HPV screening on the mean rate of negative LLETZ (false positive screening). Women who are negative for high risk HPV phenotypes have been shown to have higher rates of negative LLETZ[9-10]. Moreover, HPV testing has been shown to increase the detection of low grade squamous lesions by failing to differentiate between transient and transforming infections[15].

The objectives of this study were to evaluate whether primary HPV DNA cervical screening has reduced the mean rate of negative LLETZ histology and to examine predictors of negative LLETZ after the introduction of HPV screening.

Materials and methods;

A retrospective, single centre, comparative cohort study was completed at University Hospitals Bristol NHS Foundation Trust (a sentinel site for primary HPV screening in the UK). Ethical approval was granted by the Southwest Cornwall and Plymouth National Research Ethics Committee (study number 14/SW/0127).

Women 25–64 years old who had a LLETZ before the introduction of HPV screening (2008) and after the introduction (2011) were eligible for inclusion as two independent cohorts. Women outside of this age range are not eligible for cervical screening in England. Women who had a LLETZ during the Sentinel Sites Study (2008 – 2010)[13] were excluded as Bristol was a pilot centre for HPV triage in the UK and management of these women, including frequency and type of follow up, was different to national protocols instituted after the publication of the study. Women in the pre-HPV testing cohort were referred to colposcopy for all grades of cytological abnormalities and referrals in the post-HPV testing cohort were for a HR-HPV positive test with subsequent cytology triage. HPV DNA testing was by Hybrid Capture 2 (HC2) which gives a pooled result of high risk subtypes but not individual genotyping. Women reviewed in colposcopy before the introduction of HPV cervical screening were not managed by HPV triage.

A sample size calculation estimated 401 subjects would be needed in each cohort; assuming a negative LLETZ incidence of 14% (from Livasay *et al*[7]), a reduction in negative LLETZ of 50% (to 7%)

following the introduction of HPV screening, with alpha at 0.05 and power at 90%. The incidence from Livasay *et al* was used as they reported the mean incidence of comparable studies and an effect size of 50% was based on the improved sensitivity of HPV testing. Although the specificity of HPV screening alone is poor in young women for detecting CIN2+ (76.5%)[16], and therefore an increase in negative LLETZ may be anticipated, the women in the HPV testing cohort who were positive for HR HPV were triaged with cytology which increases the specificity to 95.6%.[17]

Participants were randomly selected from a colposcopy database by an independent Information Analyst using 'treatment type' and 'appointment date' as search terms. Individuals were selected into the study on the basis of the exposure (whether or not they had had HPV screening), not the outcome, and as such a case control design would not have been appropriate. Therefore, potential confounders such as age, smoking status, parity and contraceptive use were recorded (smoking and parity increase the risk of CIN progression[18,19]). These data were divided into categories for ease of analysis: age into 30 years or less, 31-40, 41-50 and 51 years or more; contraceptive into none, oestrogen and progesterone excess (postmenopausal, use of Depoprovera, Implanon and the Mirena Coil); parity as none, one and more than two. To reduce transference errors and improve the quality of the data collection, double data entry was completed and a table of definitions was constructed to ensure the researchers entered the same field values.

Pathology reports were reviewed for referral cytology, which was coded as Negative, Low grade or High Grade according to the revised 1986 BSCC Classification System[20], and for HR HPV status; coded as negative or positive. Biopsy results and histological diagnosis at LLETZ were categorized as negative, CIN1, CIN2, CIN3, cGIN or invasive. Margin involvement, excision depth (coded as ≤ 6mm, 7-10mm, ≥11mm) and limiting factors such as cautery effect, denudation or tubo-endometroid metaplasia were also recorded and coded as present or absent.

The definition of a positive LLETZ was any histological specimen which contained CIN of any grade, cervical glandular intraepithelial neoplasia (cGIN) or cancer[6-11]. The definition of a negative LLETZ was a histological specimen in which there was no evidence of CIN; differentiating between transient and transforming HPV infections is problematic and specimens containing no CIN should be considered as true false positives. As part of routine practice, LLETZ samples reported as negative were reviewed independently by two consultant histopathologists and additional blocks processed to confirm the absence of CIN. For the study purposes, all cytological and histological follow up of the negative LLETZ women were recorded to validate the result.

Differences in the incidence of negative LLETZ may have been affected by policy change rather than HPV testing and therefore variables that relate to this were collected. National guidelines in the UK[21] provide recommendations on intervals from cytological screening to colposcopic assessment, diagnostic standards for colposcopy and criteria for LLETZ. The indications for LLETZ were divided into those who had a biopsy prior to LLETZ (persistent CIN1 for greater than 24 months, CIN2 / 3 or cGIN) and those who had a see and treat LLETZ (no prior histology). Indications for see and treat LLETZ included a high grade cytology referral with confirmatory high grade colposcopic findings, a high grade cytology referral with a transformation zone type 3 or persistent (>12 months) low grade cytology with a transformation zone type 3.

Clinical records and colposcopic images were assessed to confirm the position of the transformation zone (TZ) in relation to the endocervical canal; as specified by the IFCPC nomenclature, this was coded as a TZ type 1 or 2 when the squamocolumnar junction (SCJ) was fully or partially visible (satisfactory colposcopy) or a TZ type 3 when the SCJ could not be visualized (unsatisfactory colposcopy)[22]. The size of the lesion (coded out of four quadrants), the interval in weeks from cytology to colposcopy and from colposcopy to treatment (coded as 0-4 weeks, 5-8 and more than

nine) and the management instituted (see and treat, biopsy, repeat cytology or conservative) were also recorded.

Statistical methods:

The incidence of negative LLETZ in the cohorts before and after HPV screening, were calculated along with the risk ratio and absolute risk difference. Confidence intervals and p-values for these risk statistics were estimated using the normal approximation. A set of Poisson regression models examined whether the association between the introduction of HPV screening and negative LLETZ was explained by differences in potential confounders between the two cohorts. We used a poisson regression rather than logistic model because a risk ratio is easier to interpret than an odds ratio when the prevalence of the outcome (negative LLETZ) is fairly high. Two models were estimated: the first adjusted for each of the potential confounders in turn and the second adjusted for all potential confounders. We also explored predictors of negative LLETZ in the HPV testing cohort. First, in unadjusted models we examined the association between each of the following with negative LLETZ; age, parity, contraceptive, cytology result, interval from cytology to colposcopy and TZ type and second, we included those variables where there was some evidence of an association with negative LLETZ ($p<0.05$). As a sensitivity analysis we also used a forward and backward model selection algorithm using cut offs for entry and removal of 0.05 and 0.1. All approaches resulted in the same final model. For all models we used robust standard errors to control for mild violations of the model assumptions. Stata v13.1 was used for all analyses.

Results:

All 802 women had complete data sets for the clinical and colposcopic variables collected. The majority of women were less than 30 years old, nulliparous, non-smokers, had a high grade cytology

referral and a visible transformation zone. A full description of the clinical characteristics is given in Table 1.

Compared to the pre-HPV cohort, more women in the post-HPV screening cohort were younger than 30 and older than 50 years and more women used contraceptive. The two cohorts were similar with respect to parity, smoking habits, referral cytology, histological limiting factors and visibility of the TZ. However, in the pre-HPV cohort the interval from cytology to colposcopic assessment was longer (mean 7 weeks, SD 4.21 vs mean 5.4 weeks, SD 4.44; $p<0.001$) and more women had an excision depth less than 7mm (Table 1). The LLETZ histology for the two cohorts, as presented in Table 1, was similar with respect to CIN, cGIN and invasion.

The criteria for LLETZ, as recommended by the NHSCSP publication no 20[21], were compared between pre- and post-HPV testing cohorts (Table 1) and between women with a negative LLETZ result (Table 2). In women with negative histology, the criteria for LLETZ, was similar between cohorts.

Incidence of negative LLETZ in the two cohorts.

The incidence of negative LLETZ was 19.9% (80/401) in the pre-HPV cohort and 13.4% (54/401) in the post-HPV screening cohort, giving an unadjusted relative risk of 0.68 (95% CI: 0.49 to 0.93); in Table 3. The largest confounder in Table 3 was the interval from cytology to colposcopy; after adjusting for this variable there was no evidence of an association between HPV screening and negative LLETZ (RR 0.83; 95% CI: 0.60 to 1.15). In the final fully adjusted model that controlled for differences in age, smoking, contraceptive use, parity, referral cytology, biopsy result prior to LLETZ and histological limiting factors, there was a 25% reduction in negative LLETZ in women who had had HPV screening.

Table 4 shows the association of a range of patient and clinical variables with negative LLETZ among women who underwent HPV screening. Based on the final model, and after adjusting for parity, the risk of negative LLETZ from a TZ type 3 was shown to be independent of a low grade or high grade cytology referral. The marginal probability of negative LLETZ with high grade cytology, based on the final model in table 4 was 6% (0.06, 95% CI: 0.04 to 0.09). Among women with low grade cytology this was 23% (0.23, 95% CI: 0.09 to 0.21). The risk of negative LLETZ, however, was noted to be highest among women with low grade cytology and a TZ type 3 (RR 10.4, 95% CI: 5.9 to 18.4, $p<0.001$). It should be noted that while this risk is high in both relative and absolute terms (+40%; 95% CI: 27 to 54%, $p<0.001$ based on an additive binomial model), only 22/401 (5.5%) women in our study had both low grade cytology and a TZ type 3. The risk of negative LLETZ was reduced among women who had confirmatory histology prior to LLETZ.

The association between clinical variables and a TZ type 3 was explored. A strong positive linear relationship was found between increasing age (compared to women who were less than 30 years old; among 31 to 40 years RR 1.26, 95% CI 0.69 – 2.29, among 41 to 50 years RR 2.72, CI 1.57 – 4.73 and age greater than 50 years RR 4.17, CI 2.41 – 7.21). Oestrogen use had a protective association compared to women who did not use any form of hormonal treatment (RR 0.25, CI 0.11 – 0.60), whilst progesterone use was not associated with a TZ type 3 (RR 0.93, CI 0.60 – 1.44). Lastly, there was weak evidence of an association between parity and a TZ type 3 (RR 0.66, 95% CI: 0.43 to 1.02, $p=0.06$).

<u>Cytological follow up in the negative LLETZ cohorts.</u>

In women who had HPV testing and negative LLETZ histology, follow up cytology was available for 45 (nine had moved from the area). 35/45 were HPV negative six months after LLETZ and none

developed dyskaryosis during the follow up period (mean 39.6 months, range 6 to 44 months). 4/45 were HPV positive at their six month test of cure; one had CIN1 whilst the remainder had negative cytology during follow up (mean 30 months). 6/45 women had dyskaryosis at their 6 month test of cure, (two high grade and four low grade) of whom two had VAIN and the remainder had HPV (mean follow up 33 months, range 28 to 36 months). In the women who had a negative LLETZ prior to HPV testing, follow up cytology was available for 68. 7/68 women had dyskaryosis at their 6 month follow up, (one high grade and six low grade): two had VAIN, one cGIN, one CIN1 and three HPV (mean follow up 7 months, range 6 to 18 months). 61/68 had negative cytology six months after LLETZ, of whom ten developed dyskaryosis (all low grade) during the follow up period (mean 66 months, range 6 to 102 months). Three of the ten women women had VAIN, one CIN2 and the remainder HPV. In summary, 3/45 (6%) women developed positive histology (CIN1+) following a negative LLETZ in the HPV testing cohort and 8/68 (11.7%) in the cytology only cohort (RR 0.59, CI 0.16 to 2.12, p=0.53).

Discussion:

Negative LLETZ is an important performance indicator in colposcopy and quality management of a cervical screening programme. The current literature has focused on HPV screening test performance and referrals to colposcopy rather than 'down line' issues of overtreatment. Our results show, for the first time, that the incidence of negative LLETZ has decreased after the introduction of HPV cervical screening, but the prevalence of false positives is still high at 13.4%. Risk factors for negative LLETZ in the HPV testing cohort were a TZ type 3, low grade cytology and parity. Women with both low grade cytology and a TZ type 3 were most at risk.

The reported incidence of negative LLETZ in women with biopsy confirmed CIN2+ varies from 5.9% to 41%[6-11]. The rates within our unit fall within this range. A histological diagnosis of CIN2 currently

mandates treatment; regression or detection of early and therefore small lesions, which may have been removed after punch biopsy, could account for negative LLETZ histology. Our findings show that between negative LLETZ cohorts there was no difference in the criteria for treatment and support evidence that colposcopic assessment and confirmatory biopsies reduce the incidence of negative LLETZ[10,11].

Missing CIN during the treatment or when interpreting the histology could account for a negative LLETZ result. Rates of positive histology following a negative LLETZ (an indicator of residual disease) were compared between cohorts; our results showed that the 95% confidence interval for the relative risk included the null value of 1 and therefore there is insufficient evidence to conclude that the groups are statistically different. The negative histology samples were assessed by two independent histopathologists with extra levels to ensure that all cases met the inclusion criteria - indicating that the same proportion of CIN should be 'missed' between cohorts. Furthermore, variables such as referral cytology, limiting histological factors and inclusion of the transformation in the LLETZ sample were assessed and found to be similar between cohorts.

The aim, following colposcopic assessment, is to treat high grade disease and allow low grade disease to resolve. If a TZ type 1 or 2 is present and CIN1 is detected, cytological follow up is recommended for 24 months[13]. The difficulty, as illustrated by our results, is when women present with a TZ type 3. The inability to visualise and histologically identify transforming infections deters conservative follow up. Moreover, a lack of national guidance in this cohort, combined with patient choice, may account for a higher rate of treatments in women with low grade cytology than anticipated.

Colposcopists may rely on the diagnostic value of HPV screening in women with a type 3 TZ to determine who requires treatment. However, the HPV DNA test currently approved in the UK is Hybrid Capture 2 (HC2) which gives a pooled result of the high risk genotypes[14]. The inability of HC2 to genotype the more aggressive subtypes, such as HPV 16 and 18 (which cause 70% of cervical cancers)[23] and the poor specificity of HPV testing for low grade cytology (which is only 86.5%)[14,24], could also be a factor in the observed rates of low grade LLETZ histology by increasing the risk of treating women who may have a transient HPV infection[15].

None the less, our results indicate that HPV testing has reduced the number of negative LLETZ specimens. HPV screening is a more sensitive cervical screening test than cytology alone for the detection of squamous cell lesions. Recent UK cervical cancer screening statistics have shown that since its introduction, the number of women referred with borderline or inadequate cytology and those with normal colposcopy has reduced. The overall proportion of women reviewed in colposcopy with CIN has increased, thereby decreasing the number with negative histology. It stands to reason that prior to HPV screening, women referred with false positive cytology would have been weeded out by a negative punch biopsy but women with unsatisfactory colposcopy and cytology reported as borderline ?high grade may have been offered treatment by anxious colposcopists, thereby increasing the rates of negative LLETZ.

Regardless of the improved sensitivity of HPV screening, our findings have shown that women with unsatisfactory colposcopy and low grade cytology are still at increased risk of negative LLETZ. To reduce false positives, studies have evaluated mechanical and pharmacological methods of improving the adequacy of the colposcopic examination. Completing an assessment at specific times during the menstrual cycle was unsuccessful[25]. The use of systemic and topical oestrogen can improve the visibility of the SCJ[26-27] but this practice has not been routinely adopted in the UK. This may be due to the side effect profile, contraindications, patient choice, patient compliance or cost of

follow up appointments. Vaginal misoprostol has had varying success (20-78.9%)[28] but patients report nausea, abdominal pain and fever. Hygroscopic cervical dilators have a reported success rate of 79-94%[29], but women with a TZ type 3 were not included in these studies. Endocervical canal curettage can be used to obtain ~1mm^3 samples of squamous epithelium from inside the cervical canal. However, the small and fragmented samples can lead to inadequacy rate of up to 19%[30-32], the inter-observer agreement is at best moderate (k = 0.58; CI 0.52 – 0.63) and diagnosis can therefore be underestimated in 16-45% of squamous cell lesions.[32-33]

In this study, double data entry and a complete data set for both cohorts removed bias caused by erroneous and missing data. To minimise residual confounding, variables which could explain the observed association between HPV testing and negative LLETZ, were collected and controlled for. HPV screening results and the colposcopic assessment were recorded before the LLETZ outcome was known and clinical data was prospectively documented, minimizing recall bias. Moreover, this study was specifically designed to address the research questions in this paper and as such, a sample size calculation along with the strict triage described above, helped ensure sufficient power.

In the pre-HPV testing cohort photographic images were not taken as part of routine practice prior to LLETZ, preventing assessment of lesion size. However local policy advocated, in both cohorts, a strict selection criteria for treatment by recommending confirmatory biopsies if a significant change in lesion size and / or grading occurred. This comparative study uses historical controls and therefore it could be argued that the decrease in negative LLETZ histology could be associated with a change in clinical practice rather than the introduction of HPV screening. To address this, potential confounders were collected and controlled for. Moreover, following the results of the sentinel sites study it would be unethical to conduct a randomized control trial when high quality studies have shown that cytology alone has poorer sensitivity for detecting CIN than HPV cervical screening.

Our results demonstrated a small association between negative LLETZ and women with high grade cytology and a TZ type 3. Currently, it appears safer at present to treat these women as more than 70% will harbour high grade CIN. The caveat to this recommendation is in women whose interval from referral cytology to colposcopy is greater than three months; repeat cytology (cytobrush and broom) in these cases may reduce false positives by as 40% of biopsy proven CIN2 and 32% of biopsy proven CIN3 have been shown to regress.[34]

There are currently no UK recommendations to guide the management of a TZ type 3 in the presence of low grade cytology[21]. The American Society for Lower Genital Tract Disorders recommends that women with low grade cytology should not be treated unless high grade CIN is detected on biopsy[35]. As endocervical curettage is not routine practise in the UK, it is difficult to implement this policy and provide histological confirmation in the presence of a TZ type 3. Our data suggests a low risk of CIN2+ in this cohort and these women may benefit from cytological follow up to avoid negative LLETZ histology and the increased risks of preterm labour and cervical stenosis.

Conclusion:

To reduce screen false positives and subsequent negative LLETZ histology, future research should focus on improving the specificity of CIN screening; this should include the use of biomarkers that detect HPV-transforming infections, HPV genotyping and the use of techniques which sample an endocervical transformation zone. Furthermore, prospective studies should assess the progression rate of CIN in women with low grade dyskaryosis and a TZ type 3 who are managed with cytological follow up. The outcome of these studies will help form the basis of treatment recommendations for women with low grade cytology, a TZ type 3 and a high risk HPV screening result.

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Author Contributions: Dr K Manley had full access to the all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Manley, Wills, Murdoch

Acquisition, analysis or interpretation of data: Manley, Wills, Morris, Hogg

Drafting of the manuscript: Manley

Critical revision of the manuscript for important intellectual content: Manley, Wills, Lopez-Bernal, Morris, Murdoch

Statistical analysis: Manley, Wills

Study Supervision: Wills, Lopez-Bernal, Murdoch

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Figure Legends:

Table 1: Description of patient and clinical characteristics of the study sample and a comparison between the pre and post HPV testing cohorts.

Table 2: Criteria for treatment in women with negative LLETZ histology in the pre- and post-HPV testing cohorts.

Table 3: The association between HPV screening and the risk of negative LLETZ (n=802). The crude association is provided along with the association after adjusting for each potential confounder and a final model adjusting for all possible confounders.

Table 4: Risk factors for negative LLETZ in women who underwent HPV screening (n=401).

Supplementary Table S1: Association between negative LLETZ (the outcome) and exposure to different variables.

Table 1: Description of patient and clinical characteristics of the study sample and a comparison between the pre and post HPV testing cohorts.

	Combined cohorts (n=802)	Pre-HPV cohort (n=401)	Post-HPV cohort (n=401)	p-value
Patient Characteristics:				
AGE:				
<30 years	350 (43.6%)	163 (40.7%)	187 (46.7%)	0.018
31-40 years	275 (34.2%)	155 (38.7%)	120 (29.9%)	
41-50 years	127 (15.8%)	65 (16.2%)	62 (15.5%)	
51+ years	50 (6.2%)	18 (4.4%)	32 (7.9%)	
PARITY:				
None	385 (48.0%)	195 (48.6%)	190 (47.9%)	0.04
1	163 (20.3%)	68 (16.9%)	95 (23.7%)	
2+	254 (31.7%)	138 (34.4%)	116 (28.9%)	
CONTRACEPTIVE:				
None	295 (36.8%)	173 (40.1%)	122 (30.4%)	0.009
Oestrogen	177 (22.1%)	79 (19.7%)	98 (24.4%)	
Progesterone	330 (41.1%)	149 (39.9%)	181 (45.1%)	
SMOKING:				
None	530 (66.8%)	251 (62.6%)	279 (69.6%)	0.15
1-5 / day	81 (10.1%)	41 (10.2%)	40 (9.9%)	
6-10 / day	91 (11.3%)	52 (12.9%)	39 (9.7%)	
11+ / day	100 (12.4%)	57 (14.2%)	43 (10.7%)	
Clinical Characteristics:				
Referral Cytology:				
Low Grade	232 (28.9%)	125 (31.2%)	107 (26.7%)	0.161
High Grade	570 (71.2%)	276 (68.8%)	294 (73.3%)	
Cytology to Colposcopy Interval:				
0-4 weeks	385 (48%)	134 (33.4%)	251 (62.6%)	<0.001
5-8 weeks	291 (36.3%)	183 (45.6%)	108 (26.9%)	
9+ weeks	126 (15.7%)	84 (20.9%)	42 (10.8%)	
TZ type:				
Unsatisfactory	130 (16.2%)	58 (14.5%)	72 (17.9%)	0.179
Satisfactory	672 (83.9%)	343 (85.5%)	329 (82.1%)	
Excision Depth:				
0-6mm	339 (42.2%)	190 (47.4%)	149 (37.2%)	0.003
7+ mm	463 (57.7%)	211 (57.6%)	252 (62.8%)	
TZ included in LLETZ:				
Yes	790 (98.5%)	397 (99%)	393 (98%)	0.38
No	12 (1.5%)	4 (1%)	8 (12%)	
Limiting histological factors:				
No	578 (72.1%)	283 (70.6%)	295 (73.6%)	0.34
Yes	224 (27.9%)	118 (29.4%)	106 (26.4%)	
Criteria for LLETZ				
Punch biopsy:		**179 (44.6%)**	**200 (49.9%)**	
Persistent CIN1 for >24 months	74 (9.2%)	40 (9.9%)	34 (8.5%)	0.16
CIN2	156 (19.5%)	79 (19.7%)	77 (19.2%)	
CIN3	138 (17.2%)	56 (13.9%)	82 (20.4%)	
cGIN	11 (1.3%)	4 (1%)	7 (1.7%)	
No prior histology:		**222 (55.4%)**	**201 (50.1%)**	
High grade cytology & TZ type 3	82 (10.2%)	24 (5.9%)	58 (14.5%)	<0.001
High grade cytology & HG colposcopy	283 (35.3%)	163 (40.6%)	120 (29.9%)	
Low grade cytology >12m & TZ type 3	58 (7.2%)	35 (8.7%)	23 (5.7%)	
LLETZ Histological Characteristics				
CIN1	85 (10.5%)	37 (9.2 %)	48 (11.9%)	0.16
CIN2	182 (22.7%)	98 (24.4%)	84 (20.9%)	
CIN3	351 (43.7%)	162 (40.3%)	189 (47.1%)	
cGIN	28 (3.4%)	13 (3.2%)	15 (3.7%)	0.86
Invasion	25 (3.1%)	11 (2.7%)	14 (3.4%)	
Negative Histology	134 (16.7%)	80 (19.9%)	54 (13.4%)	0.016

Table 2: Criteria for treatment in women with negative LLETZ histology in the pre- and post-HPV testing cohorts.

	Pre-HPV testing cohort (n=80)	Post-HPV testing cohort (n=54)	p-value
Previous Punch Biopsy:			
Persistent CIN1 for >24 months	10 (12.5%)	6 (11.1%)	0.69
CIN2	12 (15%)	5 (9.3%)	
CIN3	18 (22.5%)	13 (24.1%)	
No confirmatory histology *(See and Treat LLETZ):*			
High grade cytology & TZ type 3	9 (11.2%)	7 (12.9%)	0.97
High grade cytology & HG colposcopy	4 (5%)	3 (5.5%)	
Low grade cytology >12m & TZ type 3	25 (31.2%)	22 (40.7%)	

Table 3: The association between HPV screening and the risk of negative LLETZ (n=802). The crude association is provided along with the association after adjusting for each potential confounder and a final model adjusting for all possible confounders.

	Relative Risk (RR)	**95% CI**	**P-value**
Unadjusted:	0.68	0.49 – 0.93	0.015
Adjusted for:			
Age	0.66	0.48 – 0.90	0.010
Smoking	0.68	0.49 – 0.94	0.018
Contraceptive	0.71	0.51 – 0.97	0.031
Parity	0.70	0.51 – 0.95	0.024
Referral Cytology	0.69	0.50 – 0.94	0.02
Cytology to Colposcopy Interval	0.83	0.60 – 1.15	0.26
TZ type	0.62	0.46 – 0.84	0.002
Biopsy result	0.66	0.48 – 0.90	0.01
Excision Depth	0.69	0.50 – 0.94	0.021
Limiting histological factors	0.69	0.50 – 0.94	0.019
Adjusted for all potential confounders:	**0.75**	**0.55 – 0.97**	**0.047**

Table 4: Risk factors for negative LLETZ in women who underwent HPV screening (n=401).

	Unadjusted associations			Adjusted final model†		
	RR	95% CI	p	RR	95% CI	p
Age:						
≤30	Ref*					
31 to 40	1.36	0.69 - 2.69	0.37			
41 to 50	3.02	1.60 - 5.67	0.001			
51+	2.92	1.36 – 6.26	0.006			
Parity						
0	Ref*					
1	1.25	0.59 - 2.65	0.6	1.28	0.56 - 2.90	0.55
2+	2.87	1.62 – 5.07	<0.001	2.13	1.13 - 4.01	0.02
Smoking						
No	Ref*					
Yes	1.35	0.81 – 2.24	0.26			
Contraceptive:						
None	Ref*					
COCP	0.40	0.18 – 0.89	0.025			
Progesterone	0.77	0.44 - 1.30	0.32			
Cytology						
High Grade	Ref*					
Low Grade	3.19	1.95 – 5.21	<0.001	3.60	2.18 – 5.97	<0.001
Cytology to Colp Interval:						
0 to 4 weeks	Ref*					
5 to 8 weeks	1.61	0.92 - 2.81	0.095			
9+ weeks	2.30	1.20 - 4.42	0.092			
Colposcopy						
Satisfactory	Ref*					
Unsatisfactory	3.94	2.46 - 6.31	<0.001	2.88	1.76 – 4.72	<0.001
Biopsy prior to LLETZ:						
None	Ref*					
CIN1 >24 months	1.15	0.52 – 2.54	0.7	0.68	0.31 – 1.50	0.34
CIN2	0.25	0.08 – 0.80	0.02	0.25	0.08 – 0.79	0.018
CIN3	1.03	0.57 – 1.86	0.9	0.92	0.49 – 1.73	0.8
Excision Depth						
≥7mm	1.09	0.65 – 1.83	0.70			
Limiting Factors						
Present	1.17	0.68 – 2.01	0.60			

*Ref = Reference category

† The final model was robust to choice of selection method (forward or backwards stepwise selection) and choice of cut off for entry to, or removal from the model ($p<0.05$ or $p<0.1$).

Supplementary Table S1: Association between negative LLETZ (the outcome) and exposure to different variables.

Exposure	Negative LLETZ		P-value
	No (n = 668)	Yes (n = 134)	
HPV Testing:			
Yes	347 (51.9%)	54 (40.3%)	0.013
No	321 (48.1%)	80 (59.7%)	
Age:			
<30 years	312 (46.7%)	38 (16.2%)	<0.001
31-40 years	231 (34.6%)	44 (32.8%)	
41-50 years	92 (13.8%)	35 (26.1%)	
51+ years	33 (4.9%)	17 (12.7%)	
Smoking:			
None	446 (66.7%)	84 (62.7%)	0.246
1-5 / day	61 (9.1%)	20 (14.9%)	
6-10 / day	77 (11.5%)	14 (10.4%)	
11+ / day	84 (12.6%)	16 (11.9%)	
Parity:			
None	336 (50.3%)	49 (36.6%)	<0.001
1	143 (21.4%)	20 (14.9%)	
2+	189 (28.3%)	65 (48.5%)	
Contraceptive:			
None	221 (33.1%)	74 (55.2%)	<0.001
Oestrogen	154 (23.5%)	23 (17.2%)	
Progesterone	293 (43.9%)	37 (27.6%)	
Referral Cytology:			
Low Grade	165 (24.7%)	67 (50%)	<0.001
High Grade	503 (75.3%)	67 (50%)	
Cytology to Colposcopy Interval:			
0-4 weeks	345 (51.6%)	40 (29.9%)	<0.001
5-8 weeks	234 (35.0%)	57 (42.5%)	
9+ weeks	89 (13.3%)	37 (27.6%)	
TZ type:			
Unsatisfactory	67 (10%)	63 (47%)	<0.001
Satisfactory	601 (90%)	71 (53%)	
Biopsy prior to LLETZ			
None	353 (52.8%)	70 (52.2%)	0.03
CIN1	58 (8.8%)	16 (11.9%)	
CIN2	139 (20.8%)	17 (12.7%)	
CIN3	107 (16.0%)	31 (23.1%)	
cGIN	11 (1.6%)	0 (0%)	
Excision Depth:			
0-6mm	275 (41.2%)	64 (47.8%)	0.158
7+ mm	393 (58.8%)	70 (52.2%)	
TZ included in LLETZ:			
Yes	664 (99.4%)	126 (94.6%)	<0.001
No	4 (0.6%)	8 (5.4%)	
Limiting Factors:			
No	497 (74.4%)	81 (60.4%)	0.001
Yes	171 (25.6%)	53 (39.6%)	